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Ultrastructural study of the post-hatching evolution of the pineal gland of the chicken *(Gallus gallus)*

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Abstract. There is a marked evolution in the ultrastructure of the chicken pineal gland after hatching. The typical large follicular cavities tha tare visible just after hatching disappear with age, giving the pineal a large number of small-caliber cavities which are practically filled by the prolongations of the pinealocytes. This gives the organ an apparently solid aspect. There are still two large cell types in the follicular wall: the A and B pinealocytes, with the B type being considerably more abundant.

Introcution

The pineal of the birds occupies an intermediate position in the phylogenetic evolution of this organ. It is located between the characteristic sensorial pineals of the inferior vertebrates (fish, amphibians, reptiles) and the secretorial pineals of the mammals. Various ultrastructural studies have been made of the pineals of the birds [*Oksche,* et al., 1965, 1969, 1972], *Collin,* 1966a,b, 1967, 1968, 1969, 1971; *Fujie,* 1968; *Bischoff,* 1969; *Ueck,* 1970; *Boya and Zamorano,* 1975; *Piezze and Gutierrez,* 1975]. Although these studies are of certain value, the contributions are fragmentary because of the utilization of diverse species (magpie, sparrow, duck, dove, japanase quail, penguin, chicken) and inclusion of different ages in the same species. There are no ultrastructural studies on the morphological evolution of the pineal or the afore-mentioned authors center their studies almost exclusively on the principal pinealocyte as a cell of rudimentary sensorial character, giving little attention to the rest of the pineal components.

In our attempt of establish a normal morphological pattern for the pineal of the birds, we selected the chicken that belongs to the most utilized experimental species. In earlier studies, we described the results obtained with the optical microscope during embryonic development [*Calvo and Boya,* 1977] and post-hatching evolution [*Boya and Calvo,* 1977]; the pineal ultrastructure of the chicken was studied during its embryonic period [*Calvo and Boya,* 1977] and in the first moments after hatching [*Boya and Zamorano,* 1975]. As these studies demonstrate, the pineal parenchyma of the chicken is divided into lobules by means of conjunctive walls which leave the capsule. In the interior, the lobules are found to have a central lumen in whose walls two cellular zones or layers are clearly distinguished: the *follicular zone,* composed of pinealocytes, radially arranged around a central lumen and the *parafollicular zone* located between the follicular zone and the basal membrane of the follicle. In each of these zones, two cellular types can be distinguished: *A pinealocytes* which are the abundant cell type and *B pinealocytes,* or principal cell type, in which very rudimentary sensorial cell characteristics can be seen ultrastructurally.

In this work, we studied the ultrastructural evolution of the pineal gland of the chicken from hatching until adulthood.

Materials and methods

In the study of the post-hatching morphological evolution of the pineal gland we used of pineals chicken that were maintained under normal conditions of light and nutrition. We took samples on days 1, 2, 5, 7, 10, 15, 15, 20, 25 and 30 and also after 2, 3, 4, 7, 8 and 13 months.

The pineals were fixed in 3% glutaraldehyde in phosphate buffer or 0.1 *M* cacodylate at 7.4 pH and they were postfixed in osmium tetroxide in the corresponding buffer. After dehydration in acetone, the samples were embedded in Vestopal W. Ultrafine sections were cut on an LKB ultramicrotome and then double-constrasted with uranyl acetate and lead citrate. The examinations of the specimens were done with a Philips EM 201 electron microscope.

Results

Owing to the slow, gradual character of the post-hatching evolution in the chicken pineal, as was indicated by the results obtained with the optical microscope [*Boya and Calvo,* 1978] we prefer to dispense with the detailed description of each age period studied. In its place we have selected three representative stages of the post-hatching morphological evolution, taking as a basis for the description the most characteristic age of each stage. In this manner, the first stage, i.e. the first moments after hatching, is represented by the 2- to 5-day-old animals. The second is represented by those of 30–60 days (young chicken). In the third stage we described the ages of 8 and 13 months separately. The study of all the intermediate states has permitted us to confirm the evolutionary lines indicated in these three representative stages.

2–5 days after hatching

The ultrastructure of the chicken pineal at 2–5 days has previously been described by *Boya and Zamorano* [1975]. The morphology is similar to that found at the end of the embryonic development [*Calvo and Boya,* 1978a,b], although there are important differences. In the first place, there is a decrease in the caliber of the follicular lumina, due in large part to the occupation of the lumina by the apical projections of the pinealocytes (fig. 1). Also, there is a cellular hypertrophy which manifests itself by a decrease of the ample intercellular spaces which are typical of the embryonic period.

The pineal parenchyma has a large number of follicles which have well-defined follicular layers and very large parafollicular zones (fig. 1). In the follicular zone the B pinealocytes are clearly predominant (fig. 1). The afore-mentioned cellular hypertrophy is very manifest in this cell type. The soma contains a globular nucleus which does not exhibit the invaginations of the nuclear membrane, characteristic of the embryonic period [*Calvo and Boya,* 1979]. A basal prolongation originates in the inferior part of the soma and heads toward the parafollicular zone. These formations, along with the basal membrane of the follicles, form a fibrillar layer which will be described below. The apical cytoplasm is ample and has the characteristic organelles of this cell type (laminar lipids, mitochondria, polymorphic dense bodies; fig. 2) with the polar disposition described during embryonic development [*Calvo and Boya,* 1979]. The development of the terminal clubs, typical of the B

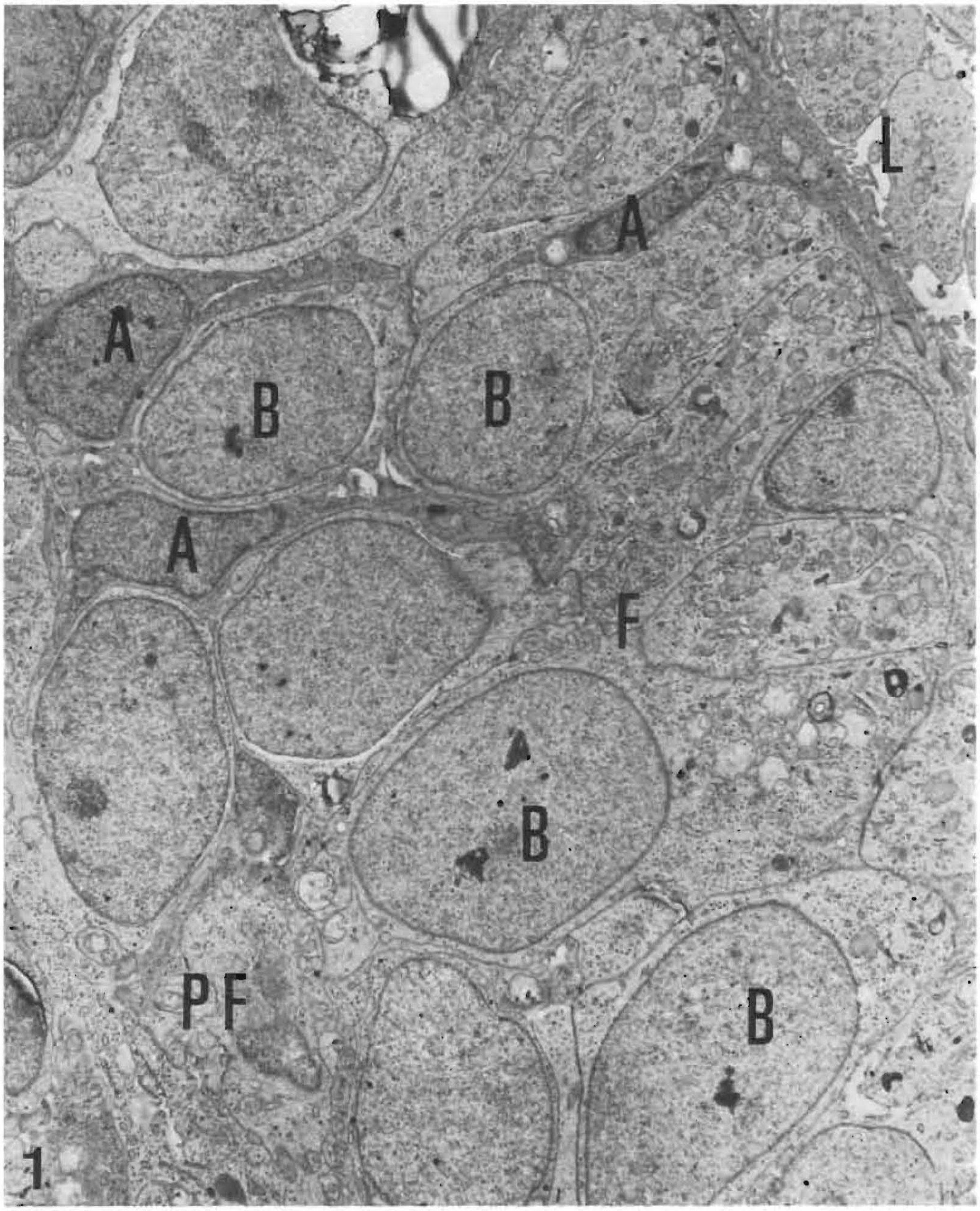

Fig. 1. Chicken, 2–5 days after hatching. Follicular wall which limits a small lumen (L), formed by the follicular (F) and the parafollicular (PF) zones; A type (A) and the B type pinealocytes (B) can be distinguished. ×4,500.

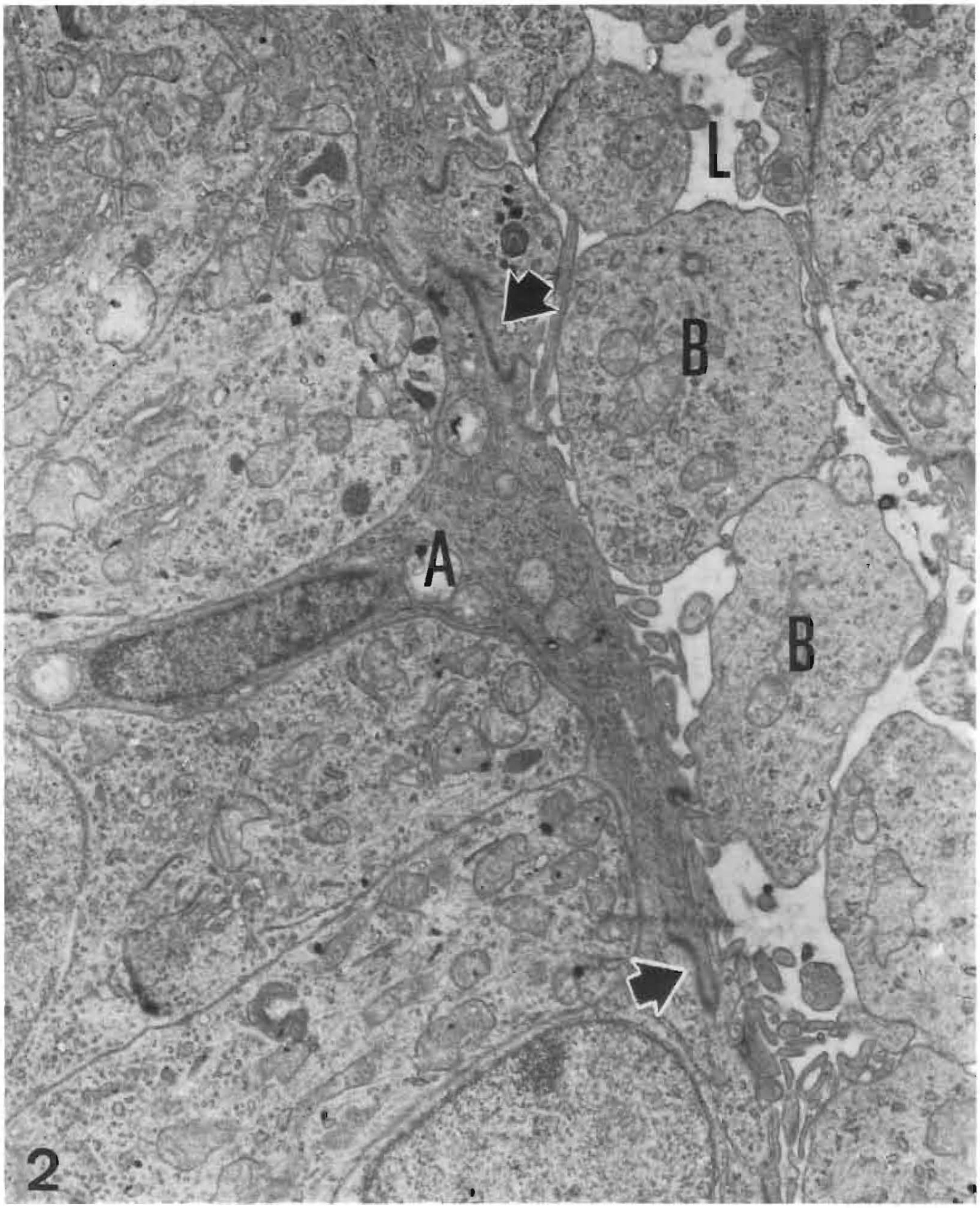

Fig. 2. Chicken, 2–5 days after hatching. Follicular lumen (L) occupied in large part by terminal clubs of the B pinealocytes (B). A pinealocytes (A) with their apical extreme extended to limit the follicular lumen, presenting union mechanisms with the adjacent cells (arrows). ×7,000.

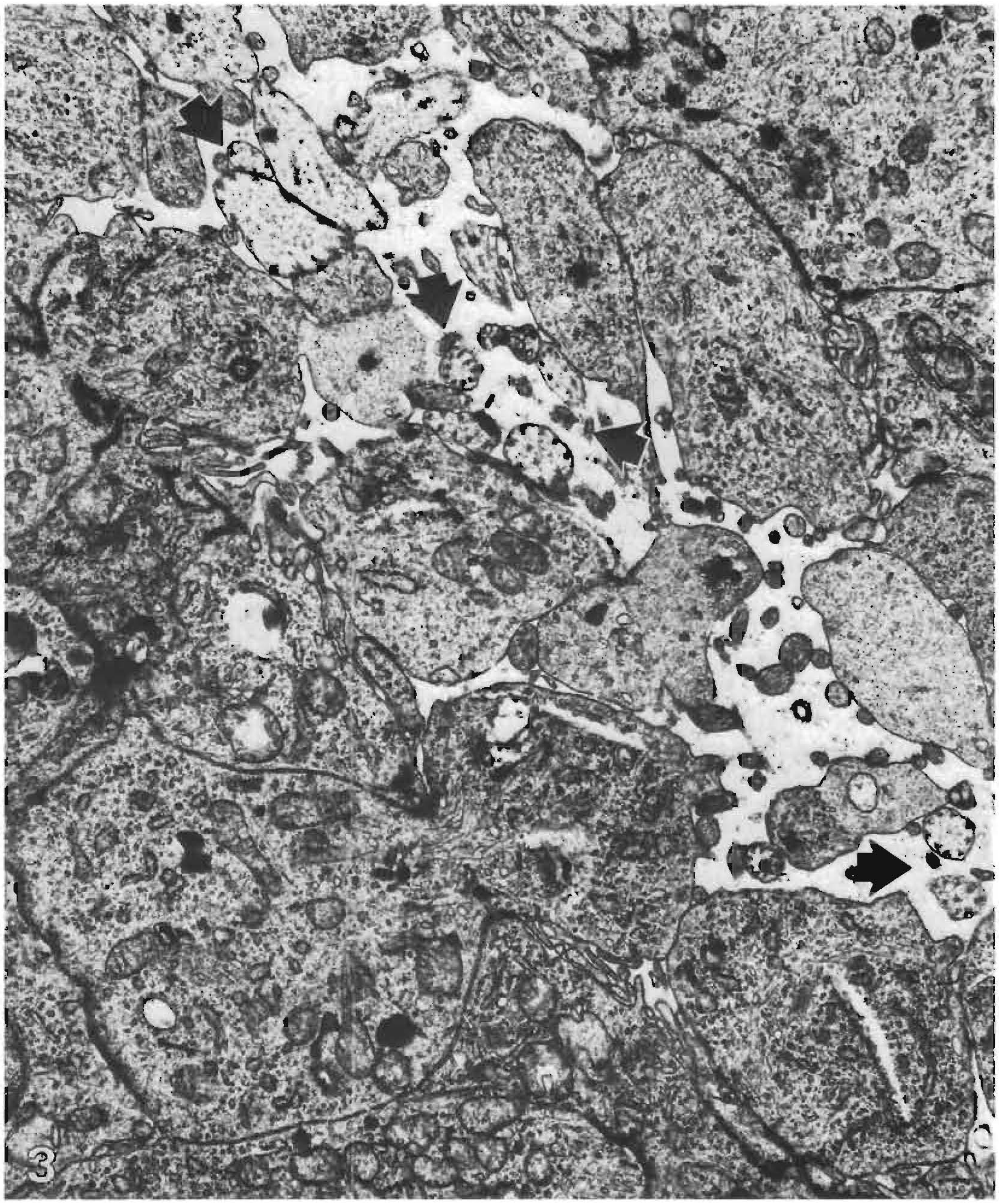

Fig. 3. Chicken, 2–5 days after hatching. Follicular lumen occupied in large part by the apical prolongations of the pinealocytes and abundant ciliar prolongations (arrows). ×7,000.

pinealocytes and which occupy a large part of the follicular lumen, is very evident (fig. 2). In the small cavities, the whole central lumen appears to be occupied by the apical prolongations of the B pinealocytes. We also encountered a large number of ciliar outlines, lacking the central pair of microtubules, in the follicular cavities (fig. 2, 3). These correspond to the cilia of the B pinealocytes. After a short trajectory, there is a bulbous dilation of the ciliar prolongation, occupied by an amorphous material in which there are no cellular organelles (fig. 3). The still unorganized ciliar microtubules, remain in the peripheral area.

There are much fewer A pinealocytes than B pinealocytes in the follicular zone. The soma of these cells is located in basal position, inside the follicular zone, although some of these cells can be located in the area in the vicinity of the lumen (fig. 1, 2). Therefore, two nuclear layers can be distinguished in the follicular zone: a superior layer, with large nuclei which pertain to the B pinealocytes, and an inferior layer close to the parafollicular zone, where the nuclei of the A pinealocytes are located. These nuclei are smaller, with more angular contours and are more electron dense than those of the B type (fig. 1, 2).

The A pinealocytes have a thin apical prolongation that has a very characteristic dilation in the vicinity of the lumen at the level of the line of the union mechanisms (fig. 1, 2). The luminal border is flat and has short, irregular microvilli and sometimes a cilium.

The cytoplasm contains the habitual organelles, although they are few in number; we note, as a characteristic of this cell type, the variable number of small, round, homogeneous lipid droplets and the round dense bodies of small caliber. The majority of the A pinealocytes possess a high-density cytoplasm which permits to distinguish them even at the lower powers of the electron microscope. However, a few cells are encountered whose nuclear characteristics and complement of organelles are very similar to those described for the A pinealocytes but their cytoplasms are only slightly electron dense. We designated these cell types respectively as the dense variety and the clear variety of A pinealocytes.

The parafollicular zone is very ample in the first moments after hatching. It is characterized by a mixture of cells and cellular prolongations without any organization. As a difference with respect to the embryonic period, the decrease and near disappearance of the intercellular spaces, characteristic of the parafollicular zone during the embryonic development may be cited [*Calvo and Boya,* 1979]. We continue to encounter numerous examples of lumina in formation in the parafollicular zone, first described by *Boya and Zamorano* [1975] and later studied during embryonic development by *Calvo and Boya* [1979].

The parafollicular zone contains the same cell types as the follicular zone, although there is no clear structural polarization except in the cells associated with a lumen in formation. The A pinealocytes pertain mostly to the dense variety. They tend to display star-shaped forms, owing to the existence of various prolongations. Very frequently, according to what has been previously described [*Boya and Zamorano,* 1975], the prolongations are associated with a formational lumen. Finally, very few A pinealocytes of the clear variety are encountered in the parafollicular zone at 2–5 days.

Immediately above the basal membrane

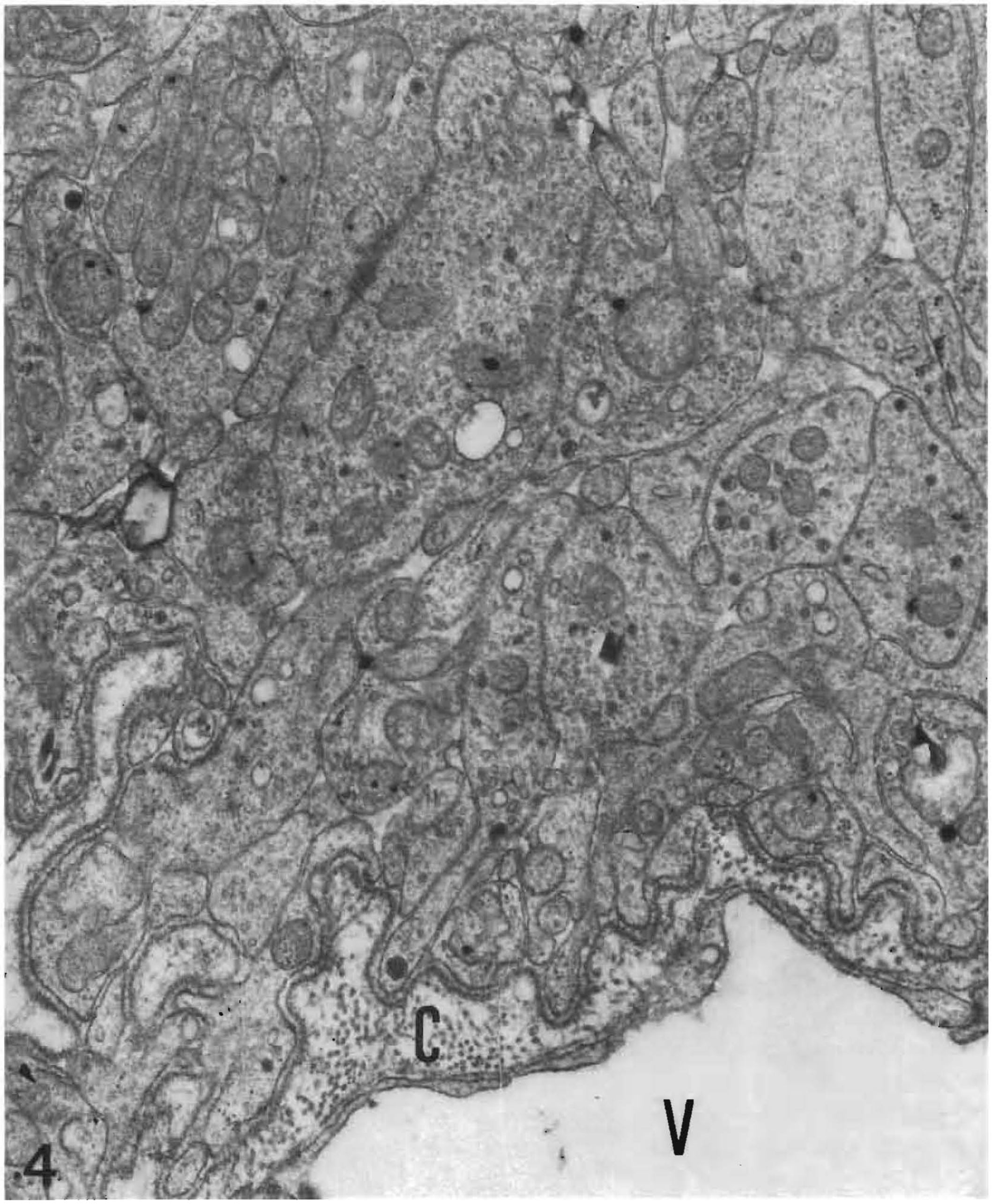

Fig. 4. Chicken, 2–5 days after hatching. Basal prolongations of the pinealocytes in the vicinity of the connective wall (C). V × Capillary. ×15,000.

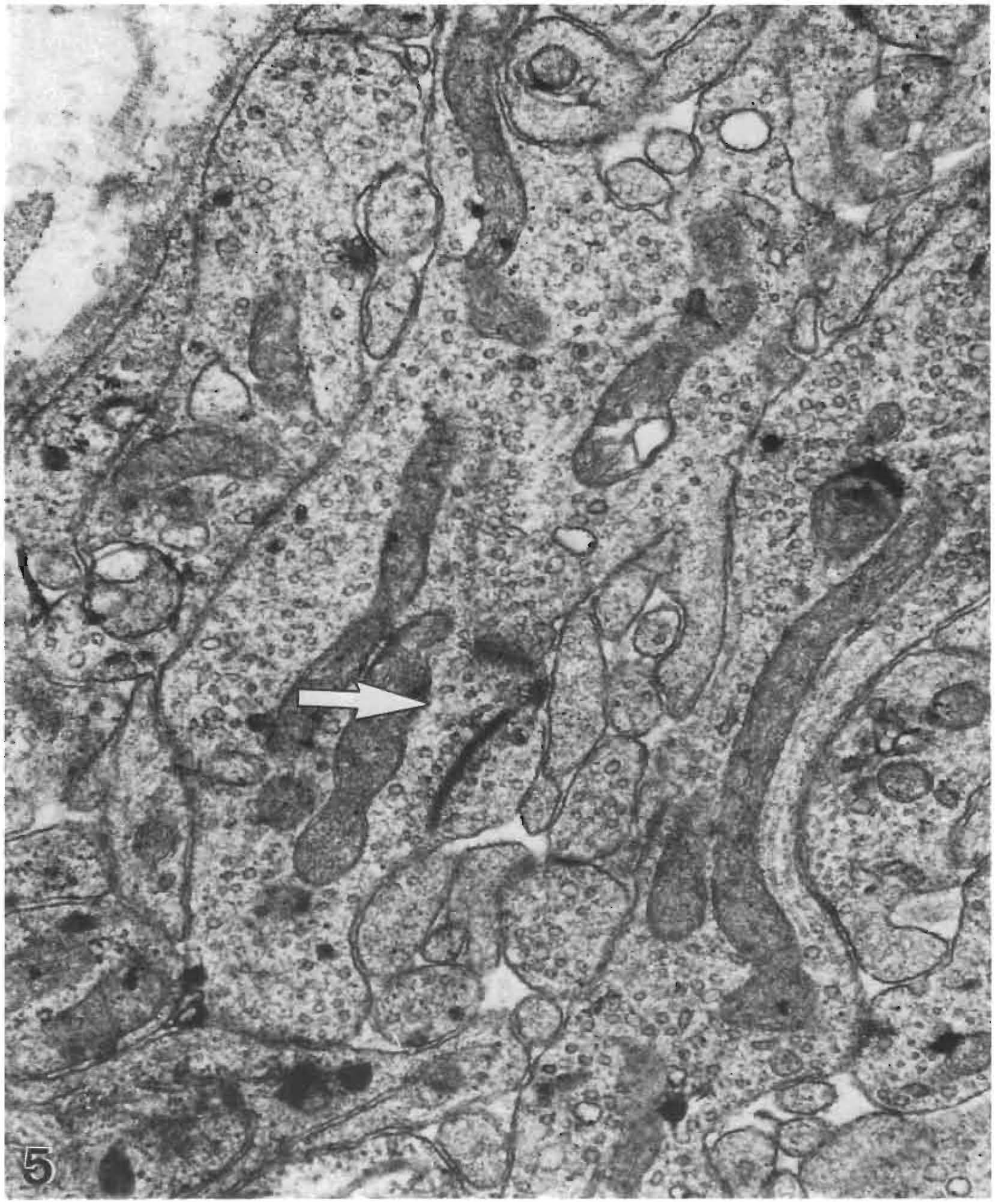

Fig. 5. Chicken, 2–5 days after hatching. Basal zone. Basal prolongations of the B pinealocytes. Synaptic ribbons (arrows). ×2,000.

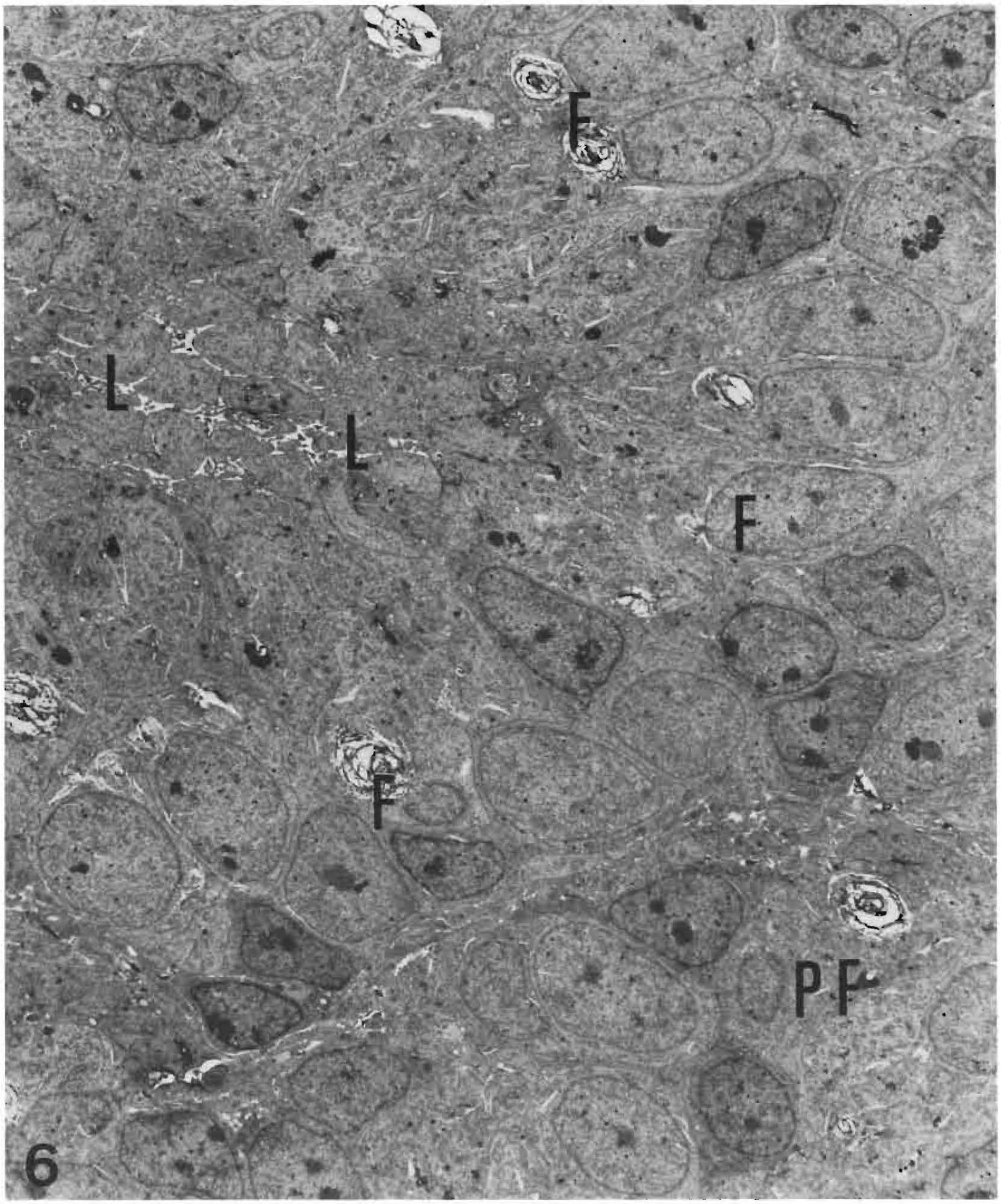

Fig. 6. Chicken, 30–60 days after hatching. Pineal follicles with minimal lumen (L) because of occupation by apical prolongations of the pinealocytes. The follicular (F) and parafollicular (PF) zones are visible in the follicular wall. ×2,000.

of the follicle, a region very rich cellular prolongations is found, this is the fibrillar layer, which contains the basal prolongations of the A and B pinealocytes (fig. 4). The first contain ribosomes, mitochondria, and, occasionally, lipid droplets. The prolongations of the B pinealocytes are characterized by their great richness in vesicles which are similar to synaptic vesicles. There are clear vesicles, granular vesicles and large vesicles whose content is transparent to electrons. The relatively frequent synaptic ribbons are a typical feature (fig. 5) in these basal prolongations. Also, microtubules and abundant mitochondria are found.

The pineal stroma in the 2- to 5-day-old chicken contains amyelinic nerve fibers which terminate in the interior of the pineal parenchyma.

30–60 days after hatching

One of the most evident ultrastructural characteristics of the pineals of 30- to 60-day-old chickens is the solid aspect the gland exhibits at low electron-microscopic magnification. At high magnification, however, a greater number of follicular lumina than that found in younger animals is visible. These lumina are of smaller diameter and are totally occupied by the apical projections of the pinealocytes, which explains the solid aspect mentioned earlier (fig. 6). The pineal stroma is denser and contains a larger quantity of amyelinic nerve fibers (fig. 7) than in the preceding stage. A special component of the pineal stroma at this age, already described with the optical microscope [*Boya and Calvo,* 1978], is the lymphoid tissue. It appears in the form of large nodules with a preferential subcapsular location whose structure is similar to that described for these formations in other organs of the birds [*Hodges,* 1974]. We also encountered numerous isolated lymphoid cells in the interfollicular conjunctival walls, as well as some migratory cells, probably of the lymphoid type in the interior of the glandular parenchyma. Nevertheless, the invasion of the parenchyma is always very limited and does not reach the massive destructive character described in other organs of the Gallinaceas [*Hodges,* 1974].

There still is an evident follicular zone around the follicular lumina. The B pinealocytes show few variations with respect to those in younger animals. There are the typical laminar lipids, in supranuclear position (fig. 6), a developed Golgi system, many mitochondria, and a large quantity of microtubules which are more abundant near the lumen. A slight increase in the number and median size of the polymorphic dense bodies may be noted (fig. 6, 8, 9). The cytoplasmic density of the B pinealocytes is variable which permits the differentiation of, at least, two types of B pinealocytes. Nevertheless, considering that the differences in the cytoplasmic densities are not very acute and especially since there is no variation in the organelles, it does not appear necessary to use a special terminology to designate these varieties.

Possibly, the most characteristic feature of the B pinealocyte is the great development of the terminal club which completely occupies the follicular lumina (fig. 8). We started to encounter, in this phase the laminar systems associated with the bulbous cilia of the B pinealocytes in the follicular lumen but in small quantity. Occasionally, we found cells in the follicular lumen (fig. 9). Their structure (nuclear morphology and organelles) was similar to that of the A

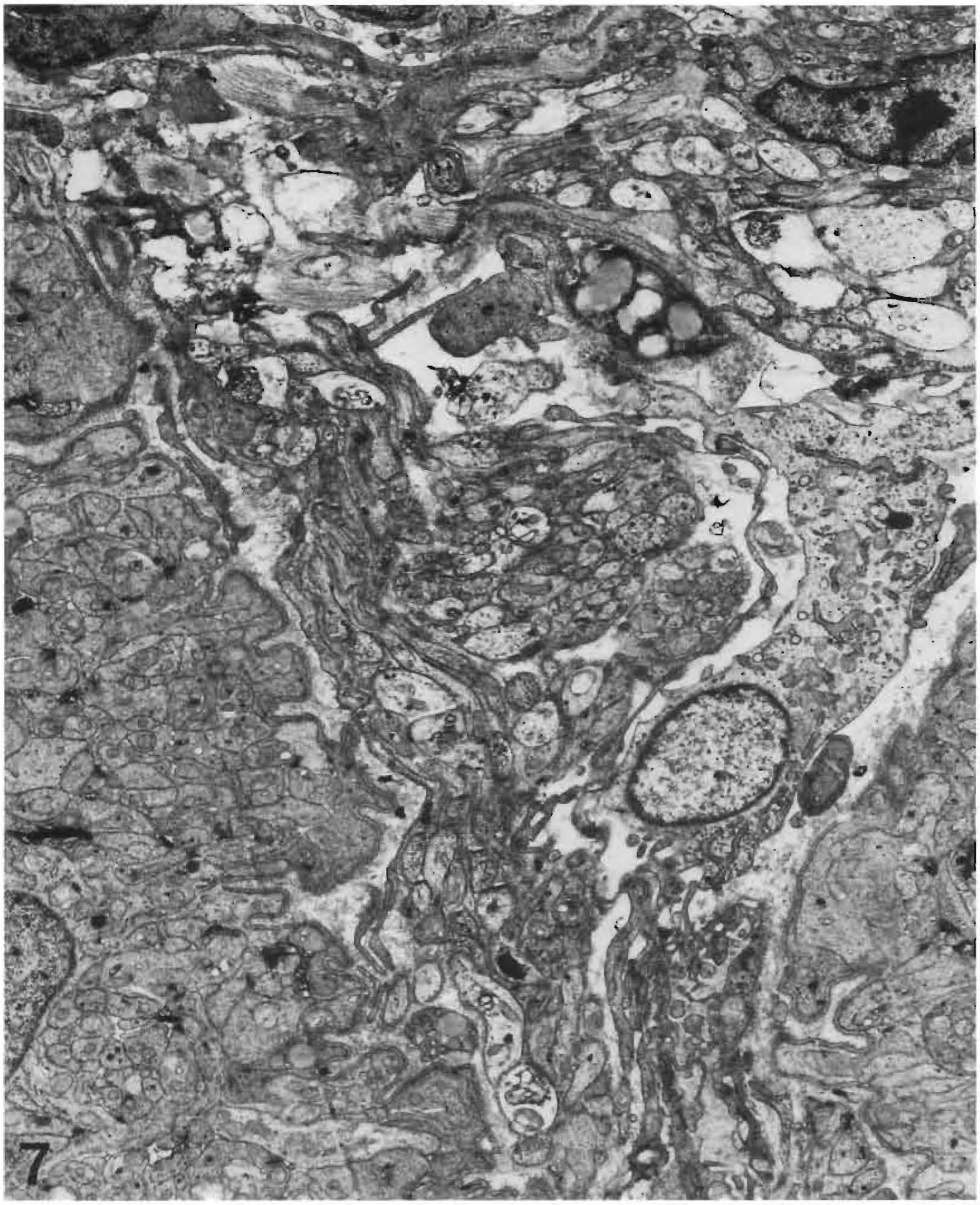

Fig. 7. Chicken, 30–60 days after hatching. Pineal stroma containing abundant amyelinic nerve fibres separated from the parenchyma by the basement membrane. ×3,000.

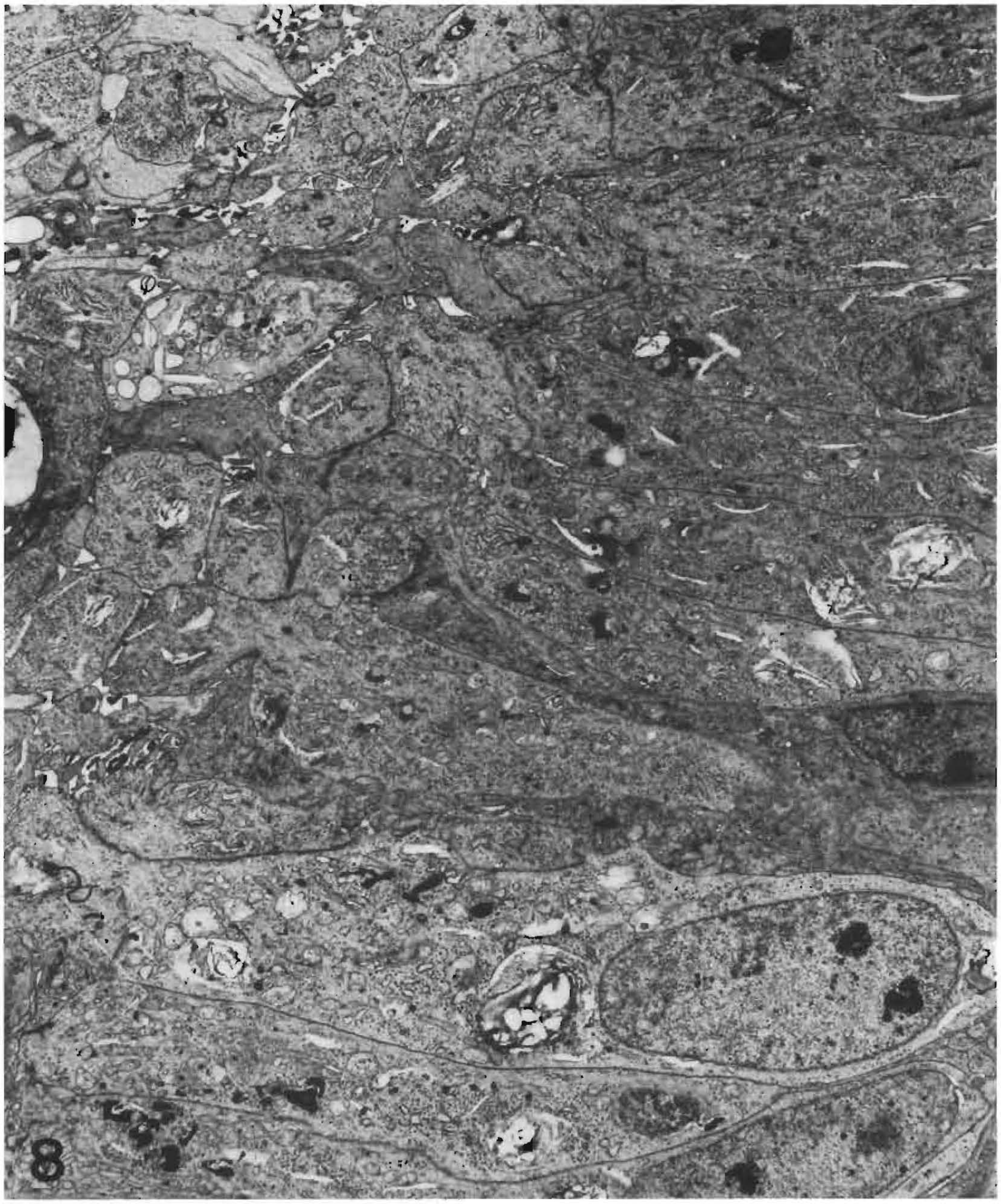

Fig. 8. Chicken, 30–60 days after hatching. Follicular zone of a wall of a follicle. Almost all the lumina are occupied by the terminal clubs of the B pinealocytes. Abundant dense bodies in this type of pinealocytes. ×3,000.

Fig. 9. Chicken, 30–60 days after hatching. Cell in the follicular lumen. The majority of this cavity is occupied by apical prolongations of the B pinealocytes. Laminar systems in the lumen (arrows). ×7,000.

pinealocytes but a definite classification of these cells was not possible.

The A pinealocytes have a larger cytoplasm which is richer in organelles than in earlier phases. At the same time, the proportion of the clear variety of pinealocytes has also increased.

In the pineal of the chicken at 30–60 days, the parafollicular zone disappears so that the cells which are left are radially ordered around the formational lumen, i.e. they adopt the characteristic disposition of the follicular zone. There are, now, very few intercellular spaces in this zone. The cells are ample and have characteristics similar to those described in the follicular zone.

8 months after hatching

All the evolutionary lines which were noted in the previous stages continue to develop. The pineal parenchyma adopts a solid aspect owing to the occupation of the follicular lumina by the terminal clubs of the B pinealocytes. In these lumina, more abundant and better developed laminar systems are more frequently encountered than in the previous stage. Cells are also found in the follicular lumen (fig. 10). The cells are radially arranged around these lumina, the parafollicular zone having almost totally disappeared. The pineal stroma is dense, rich in collagen fibrils, vessels and amyelinic nerve fibers. The lymphoid follicles continue to exist in small numbers as do the isolated lymphoid cells in the conjuntival walls. The stroma is much more irregular than in earlier phases, with numerous penetrations toward the parenchyma, which has already been described with the optical microscope [*Boya and Calvo,* 1978], but the basal membrane always maintains a fixed limit between the stroma and parenchyma. Consequently, it is more difficult to find ample zones of parenchyma than it was in the first moments after hatching.

Cytologically, the most important differences are located in the A pinealocytes. We find a predominance of the clear over the dense variety. These cells have large cytoplasms, rich in organelles. A characteristic of this cell type at this age is the 'Nebenkern' (fig. 11). They appear as large round formations, integrated with cisterns of endoplasmic reticulum without ribosomes and organized in concentric layers which are very close together. In the interior, generally in the center, there are one or more round, homogeneous lipid droplets. The 'Nebenkerne' are located in the prolongations and rarely in the somata of the A pinealocytes, especially in the clear variety (fig. 12). They are more abundant in the vicinity of the basal membrane. We encountered numerous images of the formational process of the 'Nebenkern' exclusively located in the A pinealocytes.

13 months after hatching

The structural pattern of the pineal gland in the adult chicken is completely different from that found in the embryo and in the first post-hatching phases. No ample follicular cavities surrounded by a follicular and a parafollicular zone are to be found at 13 months after hatching. In their place, the whole parenchyma displays a large number of lumina occupied by terminal clubs of the B pinealocytes (fig. 13), but they are easily identifiable when a circular line of union mechanisms is seen in the parenchyma. It is probable that each pinealocyte is in contact with one of these lumina. At this stage there is no parafollicular zone as such; all the pinealocytes are tadially positioned around

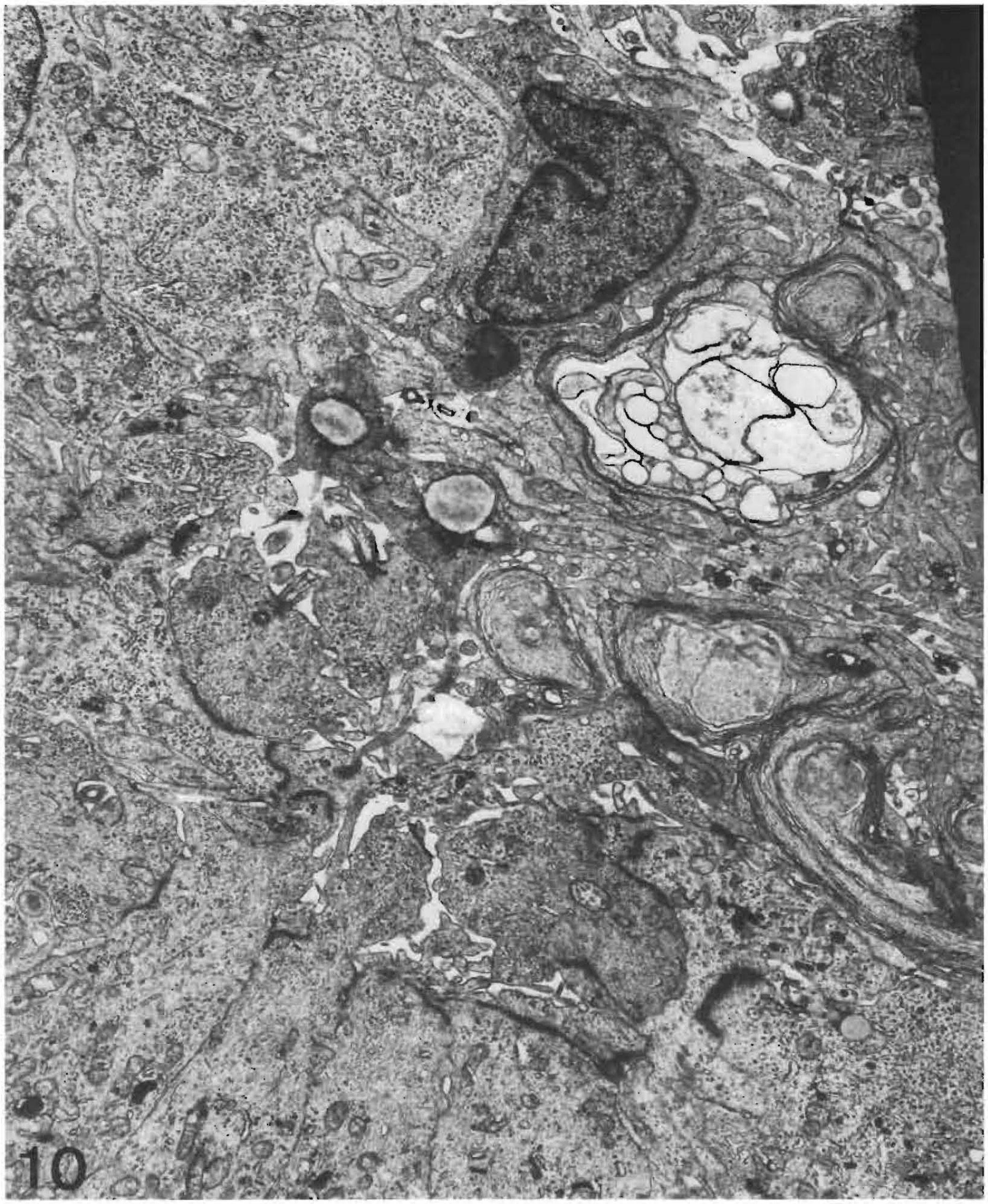

Fig. 10. Chicken, 9 months after hatching. Follicular lumen occupied by terminal clubs of the pinealocytes. Laminar systems and loosened cells. ×4,500.

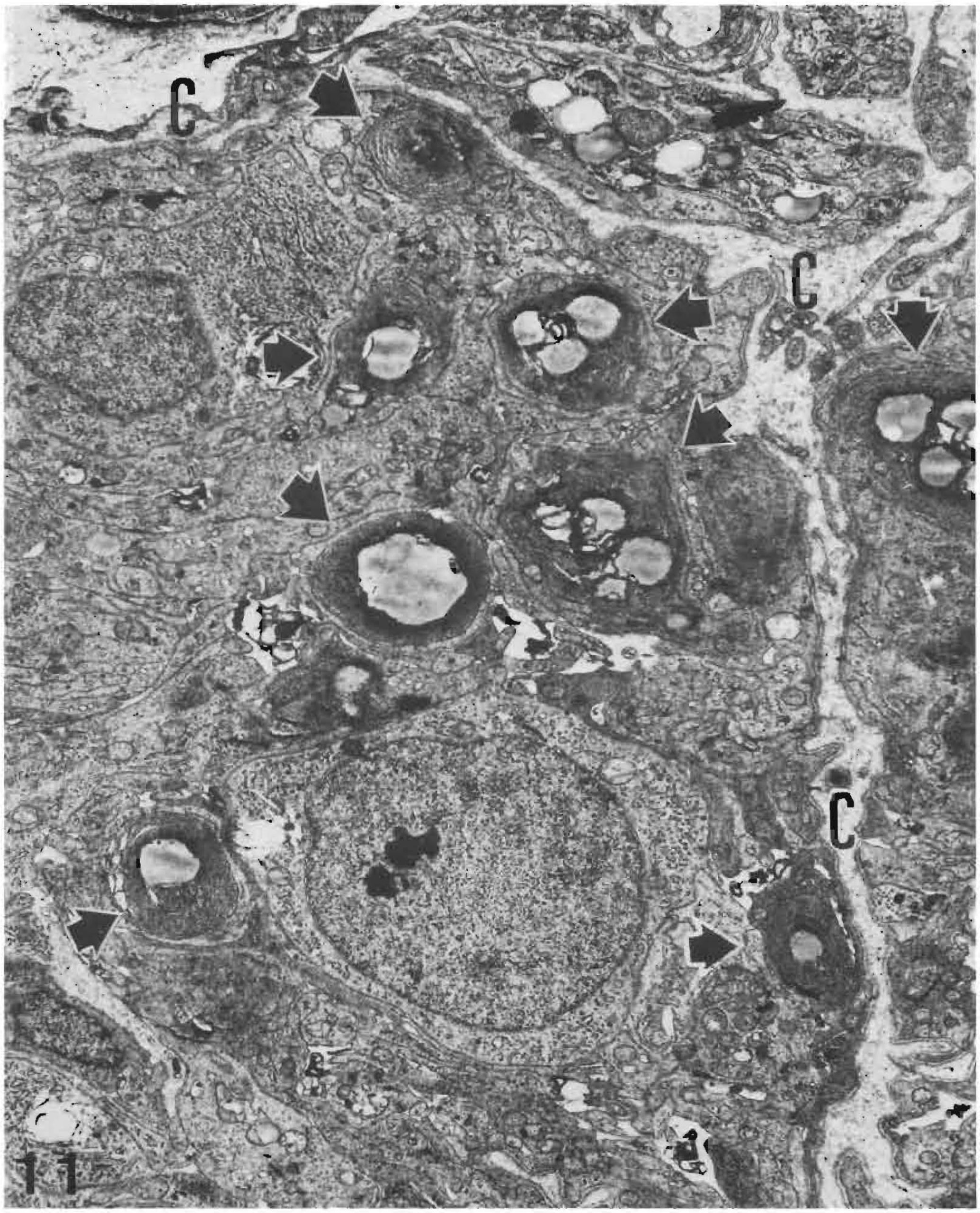

Fig. 11. Chicken, 8 months after hatching. Peripheral zone of a follicle close to a connective wall (C) in which a large quantity of 'Nebenkerne' can be seen. ×4,500.

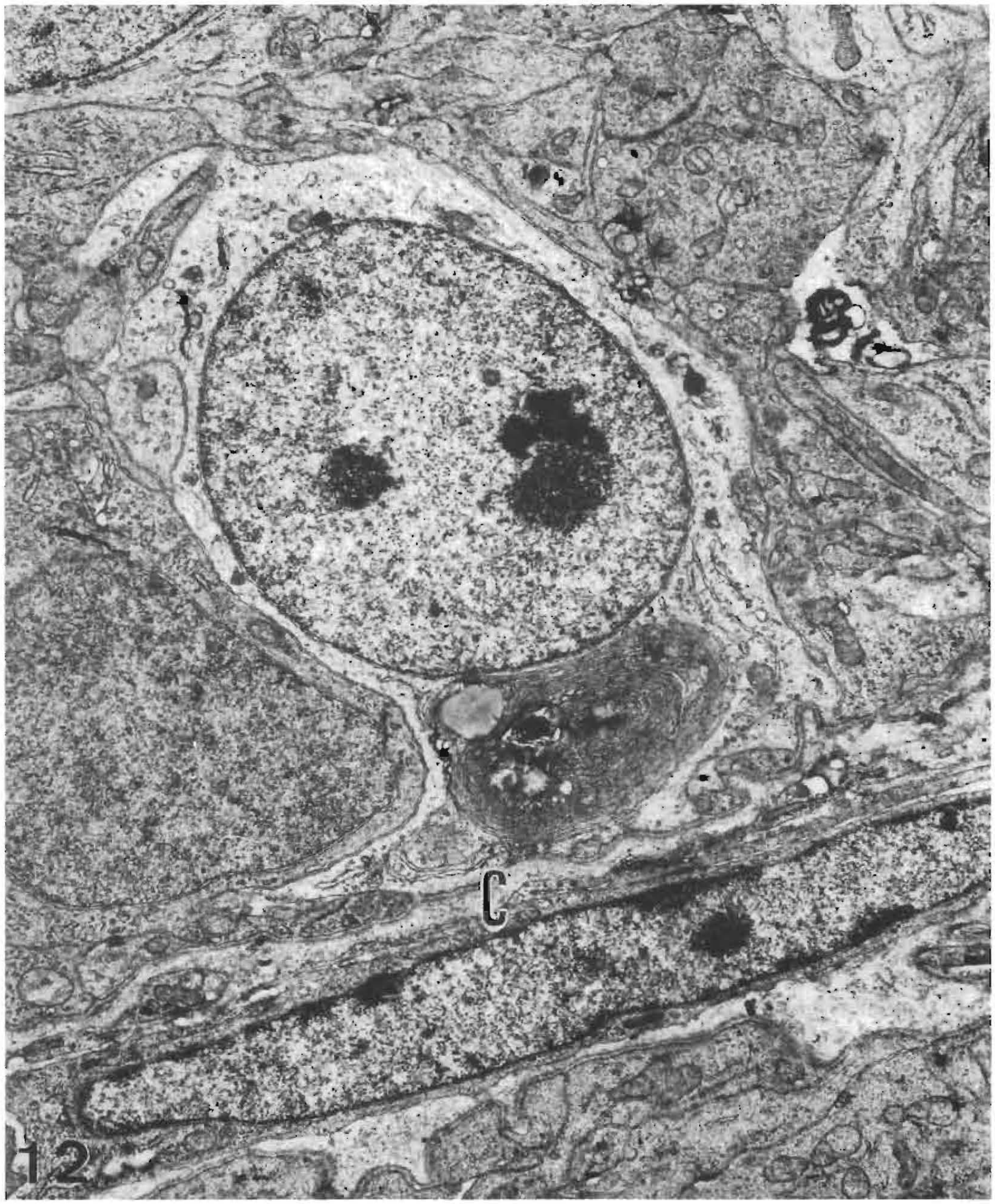

Fig. 12. Chicken, 8 months after hatching. Clear variety of A pinealocyte, next to a connective wall (C), which has a 'Nebenkern' in its soma. ×7,000.

a lumen. The penetration of the stroma toward the interior of the pineal parenchyma continues to progress. Consequently, all the pinealocytes are left a very short distance from a conjuntival wall. In many cases only a thin fibrillar layer separates the somata of the pinealocytes from the basal membrane. The pineal stroma has characteristics similar to those mentioned before but there is a greater abundance of collagen fibrils.

According to previous statements, all the pineal cavities appear occupied by apical projections of the pinealocytes. In the larger ones we find, in addition to the terminal clubs and ciliar outlines, well-developed laminar systems associated with the bulbous cilia of the B type pinealocytes. Occasionally, these laminar systems appear dilated, giving the false impression of an empty lumen. Examination at high magnification demonstrates that these cavities are limited by a trilaminar membrane instead of the usual line of union complexes.

The A pinealocyte is the least prevalent cell type of the adult chicken pineal. The majority of these cells pertains to the clear variety. Nevertheless, some of the dense varieties of cells can still be found (fig. 14), although they are very rare. In the clear variety, the development of the cytoplasm should be pointed out (fig. 15). These cells have a smaller nucleus than the B pinealocytes, with peripheral binding of dense chromatin and a clear nucleoplasm, which gives a very constrasted aspect. One or more prolongations grow from the soma toward the basal membrane, at which level some 'Nebenkerne' are occasionally found, although they are much rarer than in the previous stage. The apical cytoplasm contains a well-developed Golgi apparatus, free ribosomes, vesicles of rough endoplasmic reticulum, round lipid droplets, frequently with a dense peripheral component which is similar to lipofuscin, and abundant small, round dense bodies (fig. 15). The luminal border of these cells presents numerous, irregular microvilli, whose contents are slightly electron dense.

The B pinealocytes appear as ample globular cells with a large nucleus with lax chromatin and a dense nucleoplasm, which gives it a homogeneous aspect. They may have one or more well-developed nucleoli. The apical cytoplasm is moderately dense, more dense than the clear variety of the A pinealocytes; the density of the B pinealocyte varies slightly from one cell to another. Only rarely are laminar lipids seen in this cell type. In addition to very numerous free ribosomes we also found in supranuclear position, some short cisternae of rough reticulum. The Golgi system is not very well developed. Above this region, there is an enormous accumulation of mitochondria and large polymorphic dense bodies which tend to adopt round forms (fig. 16). Immediately below the neck (the cellular narrowing at the level of the union complexes) and at the level of the neck, practically all the cytoplasm is occupied by microtubules. In the lumen, a typical terminal club is found.

In these phases (8 and 13 months), multivesicular bodies are frequently found which have a dense matrix in all the cytoplasm of the B pinealocyte, including in the terminal club.

A basal prolongation, rich in vesicles, originates from the inferior part of the soma and heads toward the basal membrane to form part of the fibrillar layer. The ultrastructural aspect of this region is similar to that described in earlier phases.

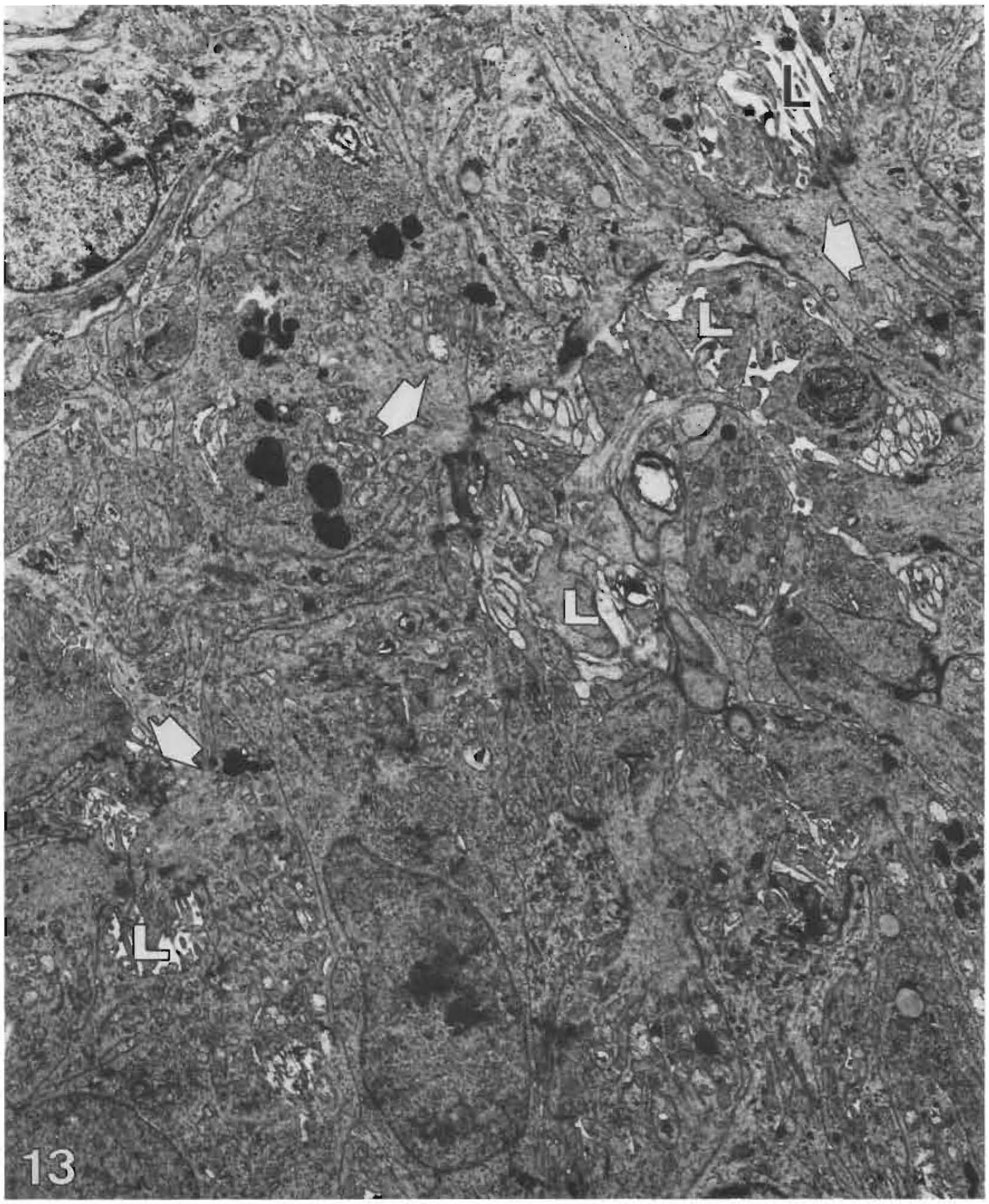

Fig. 13. Chicken, 13 months after hatching. Pineal parenchyma in which various lumina (L), practically occupied by the apical prolongations of the pinealocytes and with union mechanisms between the cells (arrows) can be seen. ×3,000.

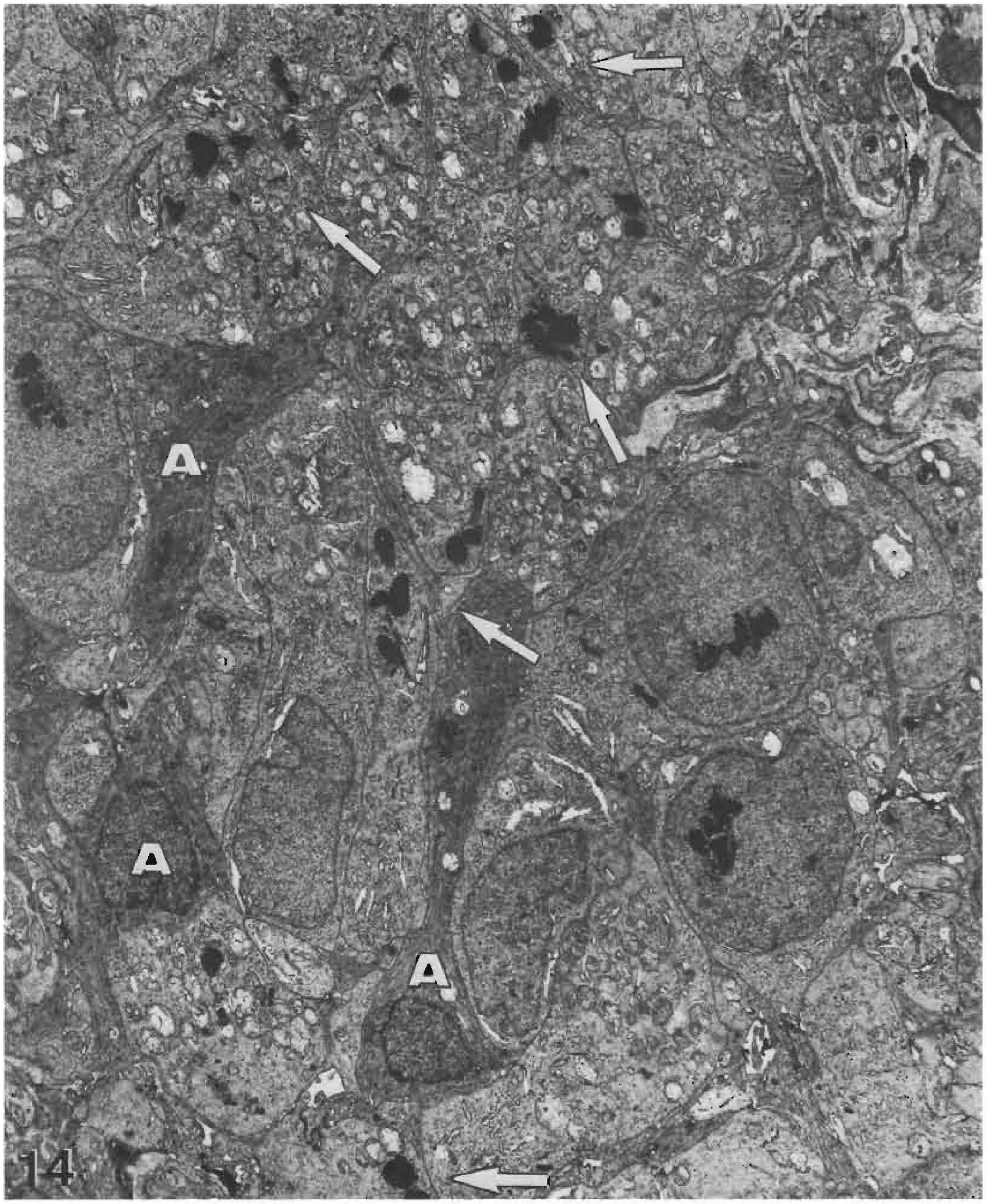

Fig. 14. Chicken, 13 months after hatching. Follicular wall with dense A pinealocytes (A) between the B pinealocytes, which are more abundant and with dense bodies (arrows). ×3,000.

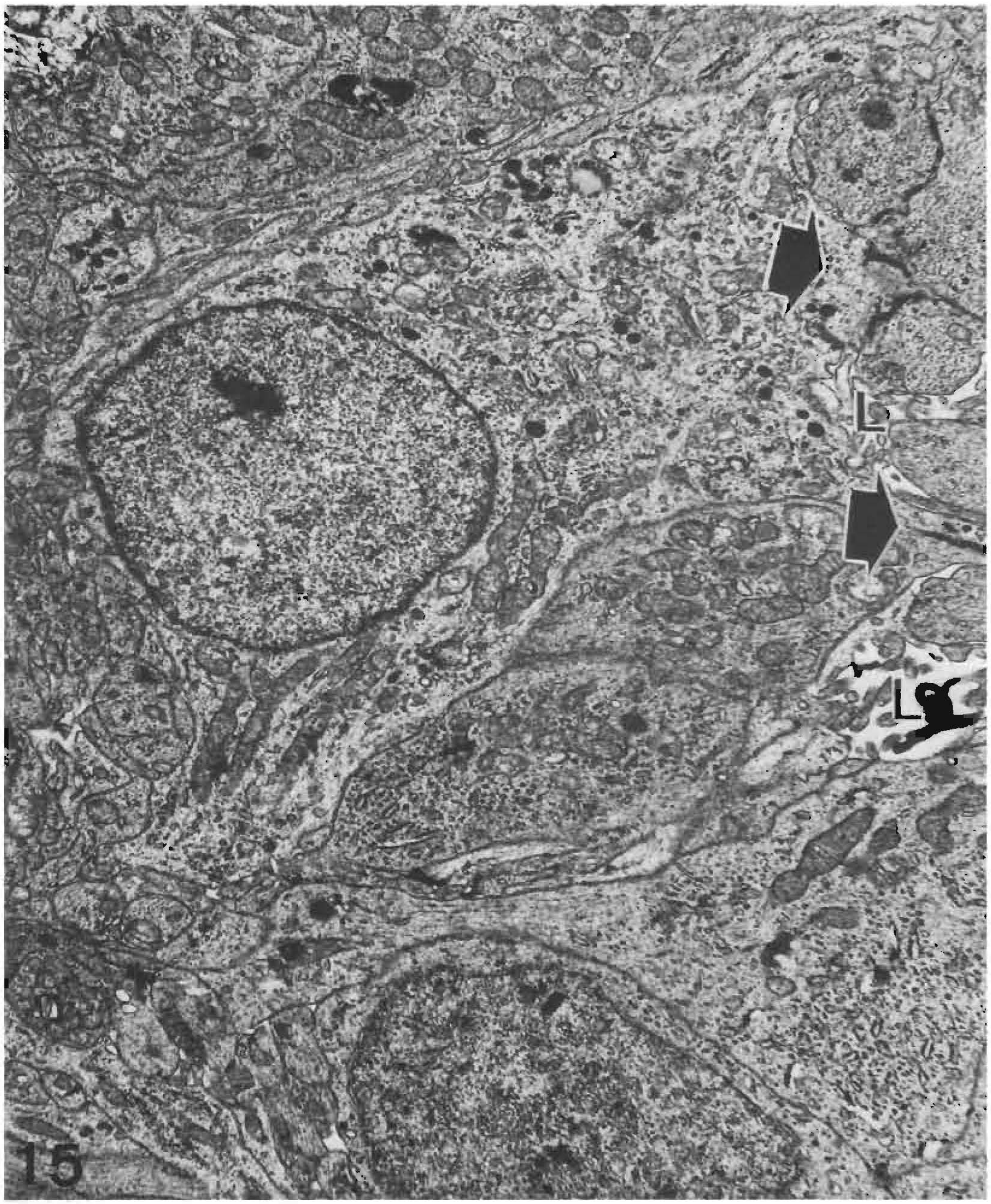

Fig. 15. Chicken, 13 months after hatching. Clear variety of A pinealocyte, with ample cytoplasm, in the follicle at whose level union mechanisms (arrows) can be seen. ×7,000.

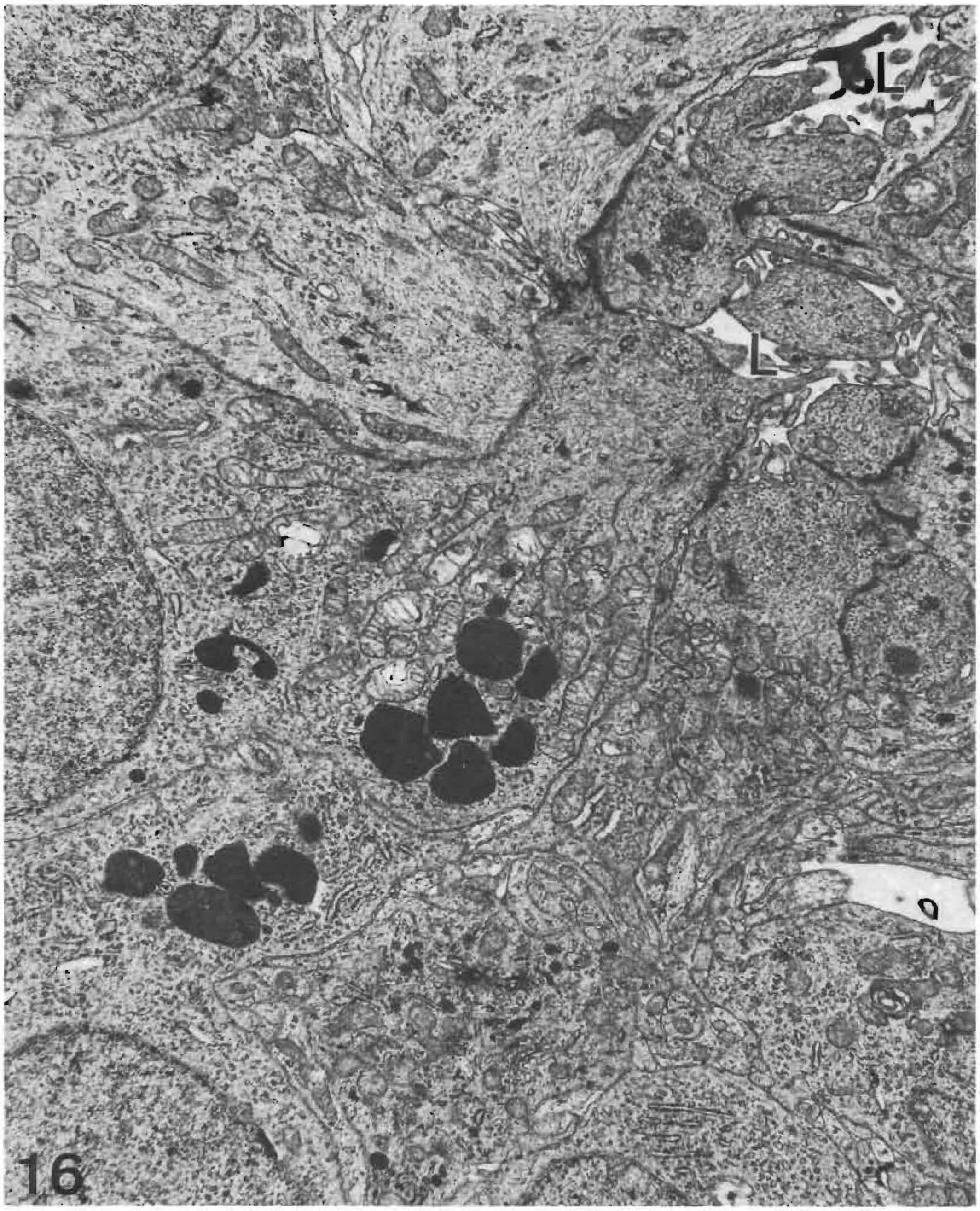

Fig. 16. Chicken, 13 months after hatching. Dense bodies in the apical portion of the B pinealocytes limit the follicular lumen (L).

Discussion

As our results demonstrate, there is a marked ultrastructural evolution of the chicken pineal after hatching. This evolution was previously studied with the optical microscope by *Boya and Calvo* [1978]. Throughout post-hatching life, variations occur in the structural pattern of the pineal gland. In the first moments after hatching, the parenchyma demonstrates a clear follicular pattern, with large follicular cavities and a thick cell wall in which the follicular and parafollicular zones are distinguishable, similar to those found in the second half of embryonic development [*Calvo and Boya,* 1978, 1979]. As the age of the chicken advances, the parenchyma exhibits a solid aspect, although a detailed examination demonstrates the existence of a large number of small lumina occupied by apical projections and surrounded by radially ordered pinealocytes. It can be shown that the majority, if not all, of the pinealocytes is in contact with one of these lumina. This structural organization is the result of an evolution which starts even before hatching and develops following four lines: (1) continuous formation of lumina in the parafollicular zone with subsequent reorganization of the pinealocytes around the lumina; (2) decrease in caliber and progressive fragmentation of the large follicular lumina formed during embryonic development; (3) occupation of the follicular lumina by the terminal clubs of the B pinealocytes, and (4) penetration of the stroma toward the interior of the pineal lobules, subdividing them into smaller territories with one or various lumina, with the corresponding radially ordered pinealocytes.

Throughout this evolution, the parafollicular zone, as such, disappears as its cells are left associated with formational lumina. One of the principal functions of the parafollicular zone is to permit this spatial reorganization of the pinealocytes. From the phylogenetic point of view, this evolution of the structural pattern of the chicken pineal is very interesting. The pineals of the embryos and those of animals in the first phases after hatching have large cavities which are reminiscent of the saccular pineals of the more primitive birds (Passeriformes) and inferior vertebrates. In the adult chicken, the parenchyma exhibits a compact aspect reminiscent of the pineal of mammals, in which vital cavities have been described, bordered by union mechanisms, which contain bulbous terminations of the pinealocytes [*Wolfe,* 1965]. From the functional point of view, this evolution is brought about by a progressive approximation of the pinealocytes to the conjuctival spaces, which favors the secretion of the pineal products into the blood.

The pineal stroma shows a progressive densification with increasing age of the chicken. A special component of this stroma is the lymphoid tissue, already studied by various authors with the optical microscope [*Romieu and Jullien,* 1942; *Spiroff,* 1958; *Quay,* 1965; *Boya and Calvo,* 1978]. We were able to confirm, by electron microscopy, the presence of this lymphoid tissue in the form of nodules, as well as isolated lymphoid cells in the conjunctival walls and even in the parenchyma proper, which has not been described previously. At present, the significance of the lymphoid tissue in the pineal gland is unknown.

The nerve fibers appear in the pineal of the chicken on the first days after hatching, increasing in later phases. Ultrastructurally, these fibers are similar to the vegetative fibers found in any other zone of the organism. They originate in the superior cervical ganglion, as has been demonstrated in the chicken by *Stamer* [1961], *Ariëns-Kappers* [1965] and *Lauber* et al. [1968]. It is admitted that these fibers terminate in the pineal parenchyma proper, although the identification of the intraparenchymal fibers constitutes a classical problem in the ultrastructural investigation of the pineal gland, not only in birds but in every other species. This is due to the presence, in the prolongations of the pinealocytes, of vesicles morphologically similar to those existing in the vegetative fibers. We think this problem can be solved only with the electron microscope using, e.g. histochemical techniques and autoradiography. Nevertheless, there are ultrastructural facts that permit a 'very probable' differentiation between the nerve fibers and the prolongations of the B pinealocytes. These cellular prolongations are very large; they have more abundant and larger mitochondria and a special type of large vesicles with an electron-transparent content; the majority of its vesicles pertain to the clear type; they possess synaptic ribbons.

The definite differentiation of the two types of A pinealocytes appears only after hatching. Although the ultrastructural image of these two varieties is distinct enough, especially referring to the electron density of its cytoplasm, they still have many characteristics in common such as: nuclear aspect, complement of cytoplasmic organelles, presence of a homogeneous type of lipids and small-caliber, round dense bodies, relationship with the follicular cavities and the same form as the luminal termination. This is the reason why we have included both varieties in the same cell type, the A pinealocyte.

The dense variety of the A pinealocyte is the most abundant in the first phases of post-hatching life. Given its constant association with formational lumina, it is probable that these cells intervene in this process. With increasing age, this lumen formation decreases progressively; at the same time a parallel decrease in the proportion of the dense A pinealocytes is observed. In the adult pineal, the majority of the A pinealocytes pertain to the clear variety and present a more differentiated cytoplasm than in earlier phases. It is not possible to determine whether the increase in the clear variety of A pinealocytes is the consequence of mitotic activity, which is low but existent after hatching [*Boya and Calvo,* 1978], or of a differentiation of the dense variety of cells, as would be indicated by the absence of signs of cellular degeneration in this last variety, which would explain their progressive disappearance with age.

It is difficult to assign a functic-ı· to a certain cell type based on purely morphological data. In other ultrastructural descriptions, the cells which would match our A pinealocytes were classified as support cells. In addition to their supportive function, the A pinealocytes may have other functions, especially the clear variety. This would seem to justify the predominance of this variety once sexual maturity has been reached (in the breed we used, complete reproductive capacity was achieved at 7 monts after hatching) and the development and richness in organelles of these cells. The functional significance of the numerous 'Nebenkern' found in A pinealocytes at 8 months of age, and their decrease in later phases remains unclear.

The ultrastructural aspect of the B pinealocytes also suffers modifications after hatching. The regional organization of the cytoplasm found in the terminal phase of embryonic development is maintained, as is the complement of organelles, characteristic of these cells. One of the most evident changes, which is already visible from the first moments after hatching, is the increase in cellular volume, which probably indicates the start of an important cellular activity. Diverse biochemical studies have demonstrated that N-acetyltransferase and HIOMT rapidly reach appropiate adult levels after hatching [*Wainwright,* 1974a,b; *Binkley and Geller,* 1975; *Binkley,* 1976]. Another important modification is the great development of the luminar prolongations of these cells.

After the first month of post-hatching life, laminar systems associated with the bulbous cilia of the follicular lumina are frequently encountered. The relationship which exists between the laminar systems and the external segments of the photoreceptors is evident. There are also studies which indicate a direct photosensitivity of the avian pineal and in enucleated animals and with superior cervical ganglionectomy [*Lauber* et al., 1968; *Munns,* 1970; *Oishi and Lauber,* 1974; *Binkley* et al., 1975; *Ralph* et al., 1975; *Zimmerman and Menaker,* 1975; *Menaker and Zimmerman,* 1976]. However, the laminar systems probably only represent vestigial structures, as most authors concede.

The development of a fibrillar layer next to the basal membrane, formed by cellular prolongations which have a great quantity of vesicles in their interior and which pertain to B pinealocytes, is very characteristic of the chicken pineal after hatching. The fibrillar layer starts to differentiate in the terminal phase of embryonic development [*Calvo and Boya,* 1975]. Mixed with the prolongations of the pinealocytes are abundant vegetative nerve fibers. The development of the fibrillar layer after hatching suggests an important function of this layer in the function of the pineal. The synaptic ribbons are a special component of the basal prolongations. These have been described by various authors in the avian pineal [*Ariëns-Kappers,* 1965; *Collin,* 1968, 1969, 1971; *Fujie,* 1968; *Bischoff,* 1969; *Ueck,* 1970; *Boya and Zamorano,* 1975]. The function of these ribbons is not known. *Ueck* [1970] is the only author who relates them with a transmissional function.

In addition to the described modifications, there are also changes in the cytoplasmic organelles of the B pinealocytes. The laminar lipids, characteristic of the embryonic period [*Calvo and Boya,* 1979] are maintained in young animals [*Boya and Zamorano,* 1975] and then markedly decrease in adult pineals. The dense polymorphic bodies undergo a notable increase in size which tends to make them rounder. Abundant multivesicular bodies are found in the apical cytoplasm of the adult pineal which were less abundant in earlier phases. All these changes indicate a clear evolution in the ultrastructural aspect of the B pinealocyte, which surely reflects a parallel evolution in the function of these cells.

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